II. RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 43 through 45 have been added. Claims 1-3, 9-15, 20-21, 24, 30-32 and 35-45 are pending, of which claims 1, 2, 9, 15, 20, 21, 24, 36and 41 have been amended. Claims 4-8, 16-19, 22-23, 25-29 and 33-34 are cancelled.

Claim 1 has been amended, and new claim 43 added, to introduce the language recommended by the Examiner at page 3 of the Action, to address the Examiner's remaining section 112 concerns. Additionally, claim 1 has been amended to refer to Applicants methodology for obtaining transgenic fish whose fluorescence is so high that they visibly fluoresce, even in the sunlight (*i.e.*, a UV light source is not required). Support for these further amendments can be found in the published specification (2004/0143864) at paragraphs [0085] (last sentence), [0086] (last two sentences), [0089] and [0090].

B. Rejections On the Basis of Alleged Non-Enablment

At page 3, the Action first rejects claims 1-3, 9-16, 24, 30-32, 35-41 and 42 under section 112, first paragraph. It is noted that the Action states that specification is enabling for the invention defined in the first paragraph of the rejection. ***Without intending to in any way concede the merits of the rejection, and specifically reserving the right to revisit this enablement rejection in future continuing applications***, Applicants have amended claim 1 and introduced new claim 43 in a manner that is believed to incorporate the essence of the language indicated by the Examiner as being directed to a fully enabled invention. Thus, it is believed that this rejection has been fully addressed. The only difference is that in light of the newly introduced obviousness rejections with respect to the color species previously found allowable over the prior art, the main claims are now broadened to include any fluorescence gene, and the

claim 1 now recites "sunlight" as the specific light source. The additional language relating to the embryos that give rise to the fish are discussed in the obviousness remarks below.

C. Rejections On the Basis of Alleged Obviousness

1. Claims 1, 9-16, 19, 24, 30-32, 35-42

The Action first rejects claims 1, 9-16, 19, 24, 30-32 and 35-42 as obvious over Higashijima *et al.* ("Higashijima"), Chalfie *et al.* ("Chalfie") and Bryan *et al.* ("Bryan," US 6,436,682).

Applicants positions with respect to the Bryan patent are already of record in our response to Office Action dated March 8, 2006, which is incorporated herein by reference.

Turning to Higashijima, it is our position that the transgenic fish produced by Higashijima fail to meet the limitations of the claims. The claims are now directed to transgenic fish that express the "fluorescent protein encoded by the gene in skeletal muscle at a level sufficient such that said transgenic fish fluoresces upon exposure to sunlight." We have discussed the support for this language above. As explained by the specification at paragraphs [0085] through [0088], a useful way to ensure that the transgenic fish have the ability to fluoresce even in sunlight is to select and use those "highly expressing" transgenic embryos that "visually [exhibit] expression of the fluorescent protein in essentially all muscle fibers in their trunk" as the embryos from which the ultimate transgenic line is prepared. The claim further states that "transgenic founders of said line fluoresce upon exposure to sunlight." Each of these matters are specifically set forth in the current main claim.

We know that the fish disclosed by Higashijima fail to meet this limitation based on an email exchange between the present inventor, Dr. Gong, and an author of the Higashijima article, Dr. Hitoshi Okamato (copy enclosed). In this email exchange, there is a first email from Dr.

Gong to Dr. Okamato, dated November 30, 2007, wherein Dr. Gong states to Dr. Okamato as follows:

> I understand that you made GFP transgenic zebrafish lines under the alpha-actin promoter and GFP is expressed in zebrafish muscle. This work was published in 1997 by Higashijima et al. Dev. Biol. 192:289-299. We made GFP and RFP ttransgenic [sic] zebrafish lines under the mylz2 promoter and they displayed strong visible fluorescent colors in adult fish even under the normal daylight. I recall that when you visited our aqurium [sic] several years ago, ***you mentioned that this visible fluorescent color was not observed in your adult fish of alpha-actin:GFP transgenic lines. You just confirmed the same information over the phone.***

(emphasis ours). In a responsive email dated December 10, 2007, Dr. Hitoshi confirmed that the actin promoter fish described in the Higashijima article did not fluoresce in sunlight.

Turning to Chalfie, this reference appears to be totally devoid of relevance to any of the foregoing issues as it simply to the fluorescent protein genes and their use. We note that Chalfie is apparently merely being cited for the proposition that fluorescent protein genes were known. Action, para. bridging pages 13-14.

Based on the foregoing, it is evident on this record that the combination of Higashijima and Bryan do not meet the limitations of the claims, and there is no other teaching of record that would teach or suggest that the production of transgenic fish that fluoresce in sunlight is desirable or even possible.

Turning to new claim 43, it is noted that this claim broadly recites the language found by the Examiner to be acceptable under 35 U.S.C. 112, first paragraph, but does not include any of the limitations discussed above. Thus, claim 43 is the new main claim, with all other claims depending from claim 43 in one way or another. With respect to claim 43 (and, hence, all claims depending therefrom), while Applicants fully maintain and reiterate the arguments with respect to Bryan as set forth in the earlier response to office action noted above, it is respectfully

submitted that the Bryan patent is not available as prior art under 102(e). As noted by the Action, the 102(e) date of Bryan is March 27, 1998. The priority date of the present application is February 18, 1999, based on the original Singapore application 9900811-2, whose disclosure it can be seen is very similar to that of the present invention. Our investigation indicates that that the present inventors had actually prepared exemplary transgenic fluorescent zebrafish in Singapore (a WTO country) with the intention of providing such fish to the ornamental fish market prior to March 27, 1998. For example, the Examiner's attention is directed to reference C35 (Gong, "Transgenic Fluorescent Fish," *Asia Pacific Biotech News*, Vol. 2, No. 16, Aug. 1998), where Dr. Gong, the present inventor, describes the fact that fluorescent zebrafish in accordance with the present invention were in existence at that time and had been prepared due to their "potential as ornamental fish in the market." While this article is after the March 27, 1998 date, Applicant's representative is currently investigating the submission date of this article as well as other documentation to support a conclusion that the present invention was made in a WTO country prior to March 27, 1998. It is thus Applicants intention to file additional evidence and an appropriate declaration shortly, as the necessary people are currently on vacation and not available.

2. Claims 2-3

With respect to claims 2-3, the Action includes the further reference of Abeywickrama et al. (U.S. 5,028,839).

Applicants first incorporate by reference the arguments set forth above.

Further, with respect to claim 3, we fail to understand how the '839 patent is relevant and how it can be said to teach or suggest "wherein the transgenic fish are displayed under an

ultraviolet light that emits light at a wavelength ***selected to be optimal for the fluorescent protein or proteins***." (emphasis ours). The Action is silent on this issue.

3. Claim 21

With respect to claim 21, the Action includes the references of Moss *et al.* ("Moss") or Chan *et al.* ("Chan").

Applicants again incorporate by reference the arguments set forth above.

With respect to Moss, while this reference does appear to disclose what appears to be a rat myosin light chain promoter and its use in driving the expression of GFP in transgenic zebrafish, it does not appear to be relevant to claim 21, which is concerned with a "zebrafish myosin light chain 2 gene promote." The fact that the Moss promoter is of rat origin can be found on page 97, col. 2 ("One disadvantage of the rat MLC-GFP construct ..." and "the rat MLC transcriptional control regions present in our construct ...").

With respect to Chan, we would note that Chan simply relates to an MLC2 promoter, but does not disclose transgenic fish comprising such a promoter, much less does it contain any disclosure relevant to the ornamental fish market or expression in sunlight.

4. Claim 20

Turning now to claim 20, it is noted that the Examiner has introduced the further reference of Liao *et al.* ("Liao"), which is cited as teaching a zebrafish MCK promoter.

First, Applicants again incorporate the arguments set forth above with respect to the primary references.

Turning to Liao, it is noted that this reference simply relates to a new method for cloning promoters, and describes the cloning of the cytokeratin ("CK") gene promoter. This is ***not*** the

zebrafish muscle creatine kinase gene promoter, so we suspect that an error has been made by the Action in this regard.

5. Claims 1, 9-16, 19, 24, 30-32, 35-42

The Action next rejects claims 1, 9-16, 19, 24, 30-32, 35-42 as obvious over Higashijima, Bryan, Yang *et al.* ("Yang") and Living Colors.

Applicants again incorporate by reference the arguments set forth above with respect to Bryan and Higashijima.

With respect to the secondary articles of Yang and Living Colors, these publications appear merely to relate to the underlying genes and neither appears to relate to fluorescent transgenic fish.

6. Non Statutory Double Patenting

The Action lastly rejects claims 1-3, 9-16, 19-21, 24, 30-32 and 35-42 over claims 1-7 of U.S. 7,135,613 on the basis of alleged non-statutory double patenting.

Applicants respectfully traverse. Applicants note that during the prosecution of USSN 09/913,898, Applicants attempted to introduce claims consistent with the claims pending in the present application. See Applicant's Amendment dated May 9, 2003. In response to this attempted amendment, the Examiner refused entry of the amendment, taking the position that such claims were found not to be drawn to the invention elected in that case, which later became the '613 patent. See Restriction Requirement mailed 12/18/03 and, again, the Restriction Requirement dated 7/30/03, which reiterated the PTO's refusal to permit Applicants to introduce claims like those here in the earlier application, as being drawn to a separate invention. Thus, the PTO has already decided that the present claims are patentably distinct. It is thus requested that the double patenting rejection be withdrawn.

CONCLUSION

Applicants believe that the foregoing remarks fully respond to all outstanding matters for this application. Applicants respectfully request that the rejections of all claims be withdrawn so they may pass to issuance.

The Examiner is invited to contact the undersigned attorney at (512) 536-3055 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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Attorney for Applicant

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Date: January 9, 2008

From: Hitoshi Okamoto [mailto:hitoshi@brain.riken.jp]
Sent: Monday, December 10, 2007 9:55 AM
To: Gong Zhiyuan
Subject: RE: GloFish matter

Dear Dr. Gong,
Our alfa-actin: GFP fish do not look green under the day light as your fish do.
Best wishes,
Hitoshi

Dear Hitoshi:
Could you give me an email confirmation about the info? I would very much appreciate your help and cooperation.
Thank you and best regards,
Zhiyuan Gong
Department of Biological Sciences
National University of Singapore
Singapore
Tel: 65-65162860
Fax: 65-67792486
email: dbsgzy@nus.edu.sg

From: Gong Zhiyuan
Sent: Friday, November 30, 2007 1:31 PM
To: 'hitoshi@brain.riken.go.jp'
Subject: GloFish matter

Dear Hitoshi:
I was nice to talk to you over the phone just a moment ago. At the request of GloFish company, may I get your confirmation for the following information we discussed?
I understand that you made GFP transgenic zebrafish lines under the alpha-actin promoter and GFP is expressed in zebrafish muscle. This work was published in 1997 by Higashijima et al. Dev. Biol. 192:289-299. We made GFP and RFP ttransgenic zebrafish lines under the mylz2 promoter and they displayed strong visible fluorescent colors in adult fish even under the normal daylight. I recall that when you visited our aqurium several years ago, you mentioned that this visible fluorescent color was not observed in your adult fish of alpha-actin:GFP transgenic lines. You just confirmed the same information over the phone.
Thanks and best regards,
(p.s. I am looking forwards to seeing you again in Feburary at the meeting in New Zealand)
Gong
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